

Oral Rotavirus Vaccines: How Well Will They Work Where They Are Needed Most?

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Rotavirus vaccines hold promise to decrease the burden of severe diarrhea in the poorest countries, where 85% of deaths due to rotavirus occur. However, the potency of live oral vaccines is lower in these challenging settings than in middle- and upper-income countries. Many hypotheses have been suggested to explain these differences that could provide clues to improve the ultimate success of these novel vaccines. Although introduction today of even moderately effective vaccines will decrease the morbidity and mortality associated with rotavirus in low-income settings, research is urgently needed to understand why these differences in efficacy occur and what could be done to improve vaccine performance to maximize the life-saving benefits of vaccination.

Rotavirus vaccines could have their greatest health benefit in the poorest developing countries in Africa and Asia where >85% of the estimated 527,000 deaths from rotavirus diarrhea currently occur [1, 2]. Two vaccines, Rotarix (GlaxoSmithKline) and RotaTeq (Merck), have demonstrated good efficacy (85%–98%) against severe rotavirus diarrhea in clinical trials conducted in the Americas and Europe [3, 4], and these vaccines have already been introduced in the routine immunization schedule of several countries in these regions. Although these vaccines have been licensed in >100 countries

worldwide, a key question remains: how well will these vaccines perform in the poorest developing countries where they could significantly decrease the number of deaths due to diarrhea? The World Health Organization (WHO) has required that the efficacy of a rotavirus vaccine be demonstrated in clinical trials in these settings, specifically in a low-income country in Africa and Asia, before it will recommend its inclusion in the global program for childhood immunization [5, 6]. In addition, the GAVI Alliance (formerly known as the Global Alliance for Vaccines and Immunization) will assist the poorest countries in financing introduction of a rotavirus vaccine if its efficacy is demonstrated in the region. Consequently, trials of Rotarix in Latin America and in Europe have led the GAVI Alliance to offer financing for vaccine introduction to poor countries in these regions, but additional evidence of efficacy will be required from trials in Africa and Asia.

Currently, trials of RotaTeq are under way in 5 low-income countries in Asia and Africa. A multicenter trial of Rotarix has been completed in Malawi and South Africa, and an effectiveness trial has just begun in Bangladesh. Immunogenicity data on Rotarix that are available from many countries representing a range of economic development indicate that responses in low-income countries are lower than those observed in middle- and high-income countries [7]. Similar data are not available for RotaTeq, because these studies are on-

Potential conflicts of interest: none reported.

Financial support: National Institutes of Health National Center for Research Resources (1KL2RR025009 to A.S.).

Supplement sponsorship: This article was published as part of a supplement entitled "Global Rotavirus Surveillance: Preparing for the Introduction of Rotavirus Vaccines," which was prepared as a project of the Rotavirus Vaccine Program, a partnership between PATH, the World Health Organization, and the US Centers for Disease Control and Prevention, and was funded in full or in part by the GAVI Alliance.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of Centers for Disease Control and Prevention or of the National Institutes of Health.

Presented in part: 8th International Rotavirus Symposium, Istanbul, 2008; and the World Health Organization Meeting on Rotavirus Vaccines, Atlanta, 2007.

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The Journal of Infectious Diseases 2009;200:S39–48

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0022-1899/2009/20009S1-0005\$15.00

DOI: 10.1093/infdis/jin103

going, and efficacy data may be available at the same time as data on immune response. Because these results of immune response could anticipate results of efficacy, in this article, we review these data and discuss possible explanations for the lower immune responses observed. Investigations to understand these issues now, before the results of all the efficacy trials become available, could help identify strategies to improve the immune response to these vaccines and perhaps their efficacy also.

EXPERIENCE WITH PREVIOUS ROTAVIRUS VACCINES IN LOW-INCOME SETTINGS

The first generation of rotavirus vaccines were monovalent animal strains that were naturally attenuated in that they did not cause clinical disease in humans but conferred protection against subsequent infection with human rotavirus strains. Clinical trials of 3 Jennerian vaccines using attenuated animal strains—G6 bovine serotype (RIT 4237 and WC3) and G3 rhesus serotype (rhesus rotavirus vaccine [RRV])—produced disappointing efficacy results in low-income settings in the Gambia, Peru, Rwanda, and Central African Republic and at American Indian Reservations [8–12]. However, in a trial of RRV among impoverished infants in Venezuela, efficacy after a single dose of RRV was 85%–90% against severe rotavirus disease [13]. This was the only trial in which the predominant circulating strain (G3) was similar to the nonhuman vaccine strain, indicating that serotype-specific protection might be of importance for the nonhuman vaccines. Although inconsistent, the first generation vaccines yielded high efficacy (82%–100%) against severe rotavirus disease in several industrialized countries [14–20], thus providing early indication of potential for differences in efficacy between developing and developed regions.

The second generation of rotavirus vaccines used the modified Jennerian approach, which includes reassortant viruses with the backbone of an animal strain that incorporate ≥ 1 human VP7 or VP4 genes. The second-generation tetravalent RRV (Rotashield; BioVirx), which was licensed and introduced in the United States but later withdrawn because of its association with intussusception, showed high efficacy in all trials conducted in developed countries [21, 22]. As with the first-generation vaccines, however, variable efficacy was observed when tetravalent RRV was evaluated in low-income settings. Initial trials of tetravalent RRV were conducted in Peru and Brazil with vaccine containing a low virus titer of 4×10^4 plaque-forming units. In Peru, efficacy against severe rotavirus diarrhea was similar after 1 (36%) or 3 doses (30%) of tetravalent RRV [23]. When the analyses were restricted to infants with severe rotavirus disease who were negative for other enteropathogens, protective efficacy was 53% for 1 dose and 18% for 3 doses of the vaccine. In Brazil, tetravalent RRV showed

protective efficacy of ~58% against rotavirus disease with >6 liquid stools per day among children vaccinated with 3 doses of vaccine, compared with placebo, at 1, 3, and 5 months of age [24]. Of interest, efficacy against this same outcome was 84% during the first season and decreased to 0% during the second season. In a reanalysis of the data from these 2 trials to assess protective efficacy with use of a 20-point severity scoring system [25], in Peru, 1 dose and 3 doses of the lower-titer tetravalent RRV had an efficacy of 64% and 19%, respectively, against severe rotavirus disease; in Brazil, the efficacy of 3 doses was 75% against very severe rotavirus disease in the absence of other enteric pathogens but decreased to 50% when cases in which enteric pathogens were present were included. The largest trial of tetravalent RRV was conducted among a poor population in Caracas, Venezuela [22]. This trial differed from those in Peru and Brazil in that a 10-fold higher dose of tetravalent RRV (4×10^5 plaque-forming units) was used, and vaccine efficacy against severe rotavirus diarrhea was 88%. The last published trial assessing the efficacy of the higher-titer tetravalent RRV among a low socioeconomic American Indian population in the United States yielded moderate efficacy of 69% against severe rotavirus disease during the first year [26]. Similar to the trial in Brazil, efficacy against severe disease during the second year decreased to 44%. Planned efficacy trials of tetravalent RRV in poor countries in Asia and Africa were abandoned when the vaccine was withdrawn from the US market because of its association with intussusception. In an immunogenicity trial in Bangladesh, tetravalent RRV showed good immune responses that were comparable to those in developed countries.

REGIONAL DIFFERENCES IN THE IMMUNE RESPONSE AND EFFICACY OF CURRENT ROTAVIRUS VACCINES

The immune response to Rotarix was compared across countries representing different income strata. The GAVI Alliance considers countries to be eligible for financial support for vaccine purchase if their annual gross national income per capita is $< \$1000$. We examined how well the geometric mean concentrations (GMCs) of IgA among infants vaccinated with 2 doses of Rotarix correlated with the income level of the country, as measured crudely by its gross national income per capita (Table 1). IgA values were available from the GlaxoSmithKline clinical trials Web site [7], and data on the per capita income were obtained from the United Nations Children's Fund [27]. We noted that infants in low-income countries had significantly lower mean GMC titers, compared with children in high-income countries (Table 2). GMC was chosen as a key integrator of immune response, because it reflects both the percentage of infants who experience seroconversion and the level of their

Table 1. Comparison of the Immunogenicity (Geometric Mean Concentration [GMC] and Seroconversion) of Rotarix among Countries by Income Status

Study location	Sample size	Study ID	GMC 1 to 2 months after second dose, ^a U/mL (95% CI)	Seroconversion, ^b % (95% CI)	GNI, US\$ per capita
Low-income countries					
All, mean value	67.3	63	...
Bangladesh	66	103992	75.3 (47.5–119.4)	66.7 (54.0–77.8)	480
India	115	103792	49.2 (36.2–66.8)	58.3 (48.7–67.4)	820
Vietnam	128	105722	77.4 (55.2–108.6)	63.3 (54.3–71.6)	690
Lower-middle-income countries					
All, mean value	90.6	75	...
Colombia	40	444563/023	88.7 (53.1–148.2)	75 (58.8–87.3)	2740
Dominican Republic	40	444563/023	42.3 (27.2–65.8)	62.5 (45.8–77.3)	2850
Honduras	38	444563/023	122.4 (71.5–209.6)	84.2 (68.7–94.0)	1200
Peru	45	444563/023	65.8 (38.3–112.8)	66.7 (51.0–80.0)	2920
Philippines	76	101555	89.7 (62.3–129.1)	78.9 (68.1–87.5)	1420
Thailand	157	103477	134.4 (104.5–172.9)	84.7 (78.1–90.0)	2990
Upper-middle-income countries					
All, mean value	106.6	72	...
Argentina	46	444563/023	142.2 (83.9–241.1)	76.1 (61.2–87.4)	5150
Brazil	35	444563/023	117.3 (68.7–200.4)	85.7 (69.7–95.2)	4730
Mexico	48	444563/023	175.1 (106.5–288.1)	89.6 (77.3–96.5)	7870
Panama	434	107077	111.7 (93.5–133.4)	73.5 (69.1–77.6)	4890
South Africa	131	444563/013	29.3 (23.0–37.3)	44.3 (35.6–53.2)	5390
Venezuela	40	444563/023	63.7 (34.9–116.4)	62.5 (45.8–77.3)	6070
High-income countries					
All, mean value	205.6	86	...
Czech Republic	182	102247	152.5 (118.9–195.4)	84.6 (78.5–89.5)	12,680
Finland	252	107876	331.8 (265.0–415.4)	90.5 (86.2–93.8)	40,650
France	83	102247	181.8 (126.4–261.6)	84.3 (74.7–91.4)	36,550
Germany	156	102247	166.0 (126.0–218.9)	82.1 (75.1–87.7)	36,620
Hong Kong	40	444563/028	314.6 (215.1–460.1)	97.5 (86.8–99.9)	31,610
Republic of Korea	48	103478	73.2 (44.5–120.3)	66.7 (51.6–79.6)	17,690
Singapore	40	444563/028	368.5 (231.0–588.0)	97.5 (86.8–99.9)	29,320
Spain	186	102247	156.3 (123.4–198.0)	85.5 (79.6–90.2)	27,570
Taiwan	35	444563/028	105.8 (67.4–166.2)	85.7 (69.7–95.2)	17,230

NOTE. Data were obtained from GlaxoSmithKline [7]. Rotarix contains $1 \times 10^{6.5}$ cell culture infective dose. The gross national index (GNI); as reported by the United Nations Children’s Fund) was <US\$975 in low-income countries, US\$976–3855 in lower-middle-income countries, US\$3856–11,905 in upper-middle-income countries, and >US\$11,906 in high-income countries. CI, confidence interval.

^a Serum rotavirus IgA antibody concentrations 1–2 months after dose 2.

^b Defined as a GMC >20 U/mL.

titer increase, but data on seroconversions show a similar trend with the countries’ per capita gross national incomes. Studies were selected in which infants received the standard 2-dose regimen and were vaccinated in a time frame consistent with standard immunization practices—generally 2 doses administered at 6 and 12 weeks of age. All laboratory analyses were conducted under well-controlled conditions so that the data could be used for regulatory approvals.

Overall, tables 1 and 2 show that GMC increases in children with the per capita gross national income in the country stud-

ied. However, substantial variability in results does exist, which may reflect local differences in the status of the populations recruited. For example, the GMCs reported from trials in South Africa and Taiwan are markedly lower than those reported in other countries with a similar gross national income per capita, and rates of GMC and seroconversion observed in Germany and Spain did not differ greatly from those observed in Panama and Brazil. However, the overall trend indicates that, despite this variability, infants from the lowest-income areas generally had GMCs and rates of seroconversion that appeared to be

Table 2. Summary of the Immunogenicity Data (Mean Geometric Mean Concentration [GMC] and Seroconversion) for Rotarix by World Bank Income Group

Income group	No. of countries	GMC 1–2 months after second dose, ^a U/mL		Seroconversion, ^b %	
		Mean	Difference from high-income group (95% CI)	Mean	Difference from high-income group (95% CI)
High	9	205.6	...	86	...
Upper-middle	6	106.6	–99.1 (–195.0 to –3.0)	72	–14 (–29 to 1)
Lower-middle	6	90.5	–115.1 (–224.0 to –5.9)	75	–11 (–27 to 6)
Low	3	67.3	–138.3 (–276.4 to –1.0)	63	–23 (–44 to –2)

NOTE. Data were obtained from GlaxoSmithKline [7]. CI, confidence interval.

^a Serum rotavirus IgA antibody concentrations 1–2 months after dose 2.

^b Defined as a GMC >20 U/mL.

lower than those seen in the more affluent countries. The reason that the immune responses appear to correlate with income level is unknown, and whether this will also correlate with a decrease in the efficacy of the vaccine will only be determined in ongoing trials.

Data on the efficacy of Rotarix is available for high- and middle-income countries, with a single study completed in a low-income setting, Malawi [28]. These data suggest a similar trend over the narrow range of results thus far. In high-income countries, both Rotarix and RotaTeq had comparable and excellent results, with efficacies of 96% and 95% against severe rotavirus disease, respectively. In middle-income settings, results are available only for Rotarix, which was slightly less effective than in high-income countries (85% in a Latin American trial and 77% in South Africa) [28–30]. In contrast, efficacy was only 49% in the poor setting of Malawi [28, 29]. Of note, in both the Latin American trial and the South African trial, there is a large spread in the income levels between and within sites; however, this variability was not reported in either study. For instance, vaccine efficacy in Mexico, one of the higher-income countries in the region and one of the largest contributors of infants in the Latin American trial, was ~95%, which suggests that many other sites might have values well below the mean (85%) for the group.

Similar differences in vaccine performance between regions of high and low income have also been demonstrated for RotaTeq. Although RotaTeq prevented 98% of cases of severe rotavirus disease in a trial conducted in Europe and the United States, vaccine effectiveness was 46% against rotavirus disease requiring hospitalization during programmatic use in the low-income country of Nicaragua [4, 31].

HYPOTHESES TO EXPLAIN DIFFERENCES IN THE IMMUNE RESPONSES AND EFFICACY OF ROTAVIRUS VACCINES IN DEVELOPED AND DEVELOPING COUNTRIES

Why should the live oral vaccines, which are highly effective in large field trials in Europe (both vaccines), Latin America

(Rotarix), and the United States (RotaTeq), not work equally well among poor children in developing countries? There are fundamental differences in the behavior of live oral vaccines in the guts of infants in these poor settings that may significantly have an impact on the degree of their efficacy. Live oral vaccines have to jump several biological hurdles between oral administration and their replication in the gut to induce an immune response and protect against disease. This problem was initially identified when oral poliovirus vaccine (OPV) was tested in India. John [32] found that the immune response to OPV in northeastern Indian children was significantly lower than that observed in western infants, which forced changes in the formulation of the vaccine. In parts of India, many children with acute flaccid paralysis have received ≥ 10 doses of OPV, which supports the view that this vaccine is unable to effectively immunize some children in this population [33]. A recently released study from India found that mucosal immunity induced by some OPV formulations was also significantly lower in the northeastern states with lower efficacy, possibly because of interference of vaccine uptake as a result of the high prevalence of diarrhea in these regions [34]. Studies of live oral cholera vaccines conducted in Thailand and Indonesia demonstrated that Thai and Indonesian adults required a 10-fold higher dose of the vaccine to achieve an immune response comparable to those in adult volunteers in the United States [35].

A critical characteristic in the development of live oral rotavirus vaccine is determination of the titer of the vaccine virus strains and the number of doses required to induce solid protection. For Rotarix, the chosen titer (1×10^6 focus-forming units) administered in 2 doses has been sufficient to yield a good immune response and a high level of efficacy and safety in European and Latin American children. For RotaTeq, the vaccine strains grow less well; thus, each of the 5 reassortants had to be individually titered to arrive at effective levels that ranged from 2×10^6 to 116×10^6 focus-forming units for the different strains. A series of 3 doses of this vaccine has been chosen for licensure. Because the immune response and efficacy of these vaccines are dose dependent, host or environmental

factors that could reduce the dose of vaccine might also be expected to reduce its immunogenicity and efficacy.

An infant's immune responses to a live oral vaccine may be influenced by both (1) factors that decrease the effective titer of the vaccine virus reaching the intestine and (2) factors that might impair the infant's host response. Three conditions that may lower the effective titer of the vaccine delivered to the gut are the levels of transplacental antibody acquired from the mother, immune and nonimmune components of breast milk, and the amount of gastric acid in the infant's digestive tract. The host response to the vaccine may be diminished by factors such as micronutrient malnutrition (eg, zinc and vitamin A), interfering flora in the gut, and the disease state of the host (eg, diarrhea and human immunodeficiency virus [HIV] infection). Finally, differences in the epidemiology of the virus and the serotype distribution of candidate strains among settings might also alter the success of the vaccine [36]. Data for each of these hypotheses will be presented, followed by potential ways that each might be addressed to improve the immune response to and the ultimate efficacy of these vaccines.

Maternal (transplacental) antibody titers. Despite the progress made in developing several rotavirus vaccines, our understanding of the mechanisms of immunity to natural rotavirus infection remain quite incomplete [37]. Local immunity in the gut has been considered to play a key role in protection, but abundant evidence also exists to demonstrate a possible role for circulating antibodies that may work, in part, by being secreted in the gut [38]. Data from early studies of vaccine failures completed 3–5 years ago in Bangladesh and South Africa could establish whether high titers of transplacental antibody in the infant could neutralize vaccine virus in the gut and inhibit a robust immune response. Unfortunately, to date, these results have neither been released by the company nor published by the investigators [39–43].

Breastfeeding practices. Three factors associated with breastfeeding could alter the effective titer of vaccine virus: the amount of neutralizing activity in breast milk, practices around the time of breastfeeding [44], and the effect of breastfeeding [45]. Breast milk contains both IgA antibodies that can neutralize rotavirus and receptor analogues (lactadherin) that can adsorb to the virus and inhibit its attachment [46]. These factors are greatest during the first days after parturition and decrease as breastfeeding progresses [47]. Nonetheless, an early study of the neutralization of a serotype 1 virus, strain Wa, in breast milk samples collected from nursing mothers in Bangladesh yielded results that may be instructive [48]. From *in vitro* studies in which breast milk was mixed with an equal volume of live virus, ~30% of the breast milk samples were able to neutralize 99% (2 logs) of virus, and 60% of the breast milk samples could neutralize 90% (1 log) of virus. These data suggest that, if an infant had breast milk in its mouth or stom-

ach at the time when the vaccine was administered, vaccine response could be diminished [49].

The effect of breastfeeding on vaccination has been explored in the large-scale field trials of Rotarix and RotaTeq in populations in high- and middle-income countries. No differences in vaccine efficacy were noted between mothers who self-reported breastfeeding, compared with those who did not. However, these studies did not examine a key parameter critical to determining whether breastfeeding might make an impact: the specific interval between breastfeeding and the administration of vaccine [3, 4]. If the vaccine was administered at a time when the infant had not recently been feeding, the vaccine strain could pass unimpeded to the gut and begin replicating. However, if the infant was being breast fed at the time of vaccination, its mouth and gastrointestinal tract could become *in vivo* sites for virus neutralization and the vaccine might be expected to have a diminished effect. Other factors in breast milk might either buffer the virus from stomach acid and enhance its effect [49] or entrap virus and prevent it from attachment. No studies have been conducted to date to determine whether the interval between breastfeeding and vaccination has any effect on the immune response to the vaccine.

An early meta-analysis of studies with the earlier RRV suggested that breastfeeding could diminish the immune response [50]. In 18 studies of earlier rotavirus vaccines, investigators asked mothers whether they were breastfeeding at the time of vaccination. In 15 of the 18 studies, infants whose mothers self-reported breastfeeding demonstrated a lower rate of seroconversion than did those whose mothers did not self-report breastfeeding. This association was not significant in any individual study but was highly significant for the group, and breastfeeding appeared to reduce the immune response to the vaccine by ~10% in infants, compared with formula-fed infants, in the United States [50, 51]. More recently, a Thai study identified a depression of the immune response to Rotarix (96% vs. 81%) in infants who received the vaccine with no food, compared with those administered the vaccine with food, although the difference was not significant given the small sample size [45].

Stomach acid. Rotavirus can be damaged by low pH in the stomach, and each of the vaccines is administered with a buffer solution to neutralize stomach acid and maintain the titer of the virus [52]. The quantity of stomach acid in infants in developing countries has not been measured; thus, it is unclear whether a difference in levels of gastric acidity might influence vaccine uptake.

Micronutrient malnutrition. The importance of micronutrient malnutrition in enteric infections has been well studied among children in developing countries. Vitamin A supplementation is practiced in many communities in developing countries as a key nutritional intervention with a positive im-

impact on gastrointestinal function [53]. A multicenter study conducted by the WHO demonstrated that administration of zinc to infants in prospective fashion can decrease the rate and severity of diarrheal disease and even mortality in low-birth-weight infants [54]. A recent meta-analysis of randomized controlled trials to elucidate the role of zinc supplementation to prevent childhood diarrhea and respiratory illnesses revealed that zinc supplementation significantly reduced the frequency and severity of diarrhea and respiratory illnesses and the duration of diarrheal morbidity [55]. Zinc is a cofactor in many immunologic pathways, and deficiencies could influence the immune response to a viral vaccine that requires replication to prevent enteric infection. To assess the impact of zinc supplementation on the immunogenicity of a rotavirus vaccine, we conducted a trial in Bangladesh that compared the immune response to RRV (Rotashield) given with and without zinc supplementation [56]. The immune response to Rotashield (~80%) in the group that did not receive zinc was greater than expected; therefore, the study was not adequately powered to assess whether zinc might have significantly improved this response. For the Rotarix vaccine in a similar setting, the low immune response (~55%) might be improved with the simultaneous administration of zinc—a hypothesis that would be easily testable.

Interfering gut flora. Several studies have documented a lower immune response to the first dose of a rotavirus vaccine in infants who received a simultaneous dose of OPV, compared with infants who did not receive a simultaneous dose of OPV. This decreased immune response was overcome with subsequent vaccinations and has not affected efficacy or overall outcome [57]. When OPV was administered to infants with diarrhea, the infants demonstrated an impaired immune response, which lends support to the hypothesis that the immune response to a live oral rotavirus vaccine might be diminished in the presence of diarrhea or pathogens causing asymptomatic infection [58, 59]. In addition, small bowel bacterial overgrowth impaired the immune response to live oral cholera vaccine in children in Chile [60]. These observations suggest that co-inhabiting bacteria and viruses could decrease the immune response to live vaccine viruses [61]. This is of interest because infants in developing countries, in contrast to those from developed countries, may have an abundance of enteric pathogens, including enteroviruses, that are not members of the normal gut flora in their gut by 3 months of age (M. Pallansch, personal communication). To alter gut flora, Finnish investigators administered *Lactobacillus* GG to infants and documented an improved immune response to the Rotashield vaccine, suggesting a role for a balance in enteric flora in determining immune response to the vaccine [62].

Other medical conditions. Infants in Africa and Asia may also have an abundance of other acute and chronic conditions

that could impair their immune response to a rotavirus vaccine, such as HIV infection, malaria, diarrhea, and fever. For example, Gambian children with fever and malaria were found to have substantially lower serum antibody responses to *Haemophilus influenzae* type b conjugate vaccines, compared with their healthy counterparts [63]. As recommendations for the use of rotavirus vaccine were being formulated in the United States, data were unavailable to address these questions regarding efficacy among children with underlying medical conditions. This information is critical for children in developing countries.

Differences in the epidemiology of rotavirus. The epidemiology of rotavirus infection is strikingly different between infants in developing countries and infants in the United States (Table 3) [21, 61]. The force of rotavirus infection in developing countries appears to be greater, because children in these countries develop most of their severe disease in their first year of life. They may be infected year-round rather than only during the cooler winter months, often simultaneously with several different serotypes of rotavirus, and frequently harbor unusual strains, some of which represent animal-human reassortants [64]. These differences may play a role in immune response as vaccine failures are analyzed. For example, already, several reports have appeared of infants in Brazil who were vaccinated with Rotarix but became ill with serotype G2 strains [65]. Whether these illnesses are a failure of the vaccine or a natural shift in strains unrelated to vaccine is currently under investigation [66].

POSSIBLE INTERVENTIONS

This inventory of factors that can influence the immune response and efficacy of oral rotavirus vaccine provides some clear direction for research (Table 4). Additional studies to understand the relatively modest immune response observed to date and plans to improve efficacy are imperative while anticipating results of the ongoing vaccine trials.

To effectively increase the titer of vaccine that reaches the infant's gut, interventions to counteract the major inhibitory impact of high titers of maternal antibody must be considered. Two approaches seem possible: the approach used for oral chol-

Table 3. Differences in the Epidemiology of Rotavirus

Variable	Developed countries	Developing countries
Seasonality	Winter	Year-round
Age <1 year, %	40	80
Serotypes	5 Common	Many uncommon serotypes and reassortants
Mixed infection	Rare	Common
Case fatality	Low	High

Table 4. Possible Interventions to Improve the Immune Response to Rotavirus Vaccines

Intervention	Rationale	Comment
Delay breastfeeding at time of vaccination	Antibodies in breast milk can neutralize effective titer of vaccine and impair (lower) immune response	Optimal interval between vaccination and breastfeeding is unknown; feasibility will require consideration
Delay vaccination	High titer maternal antibodies can inhibit immune response; delay of vaccination could improve efficacy	Delay could increase risk of intussusception and place infants at risk for natural rotavirus infection
Increase vaccine titer or increase number of doses	Increased titer of maternal breast milk antigens and maternal serum antibody can inhibit vaccine uptake	Changing dose and/or schedule would require additional trials, expense, and time
Zinc supplementation	Zinc deficiency can impair immune response or viral clearance	May not be efficacious in all settings
Probiotic supplementation	Bacterial and viral agents in gut prevent attachment of vaccine; probiotics may restore flora, thus optimizing vaccine take	May not be efficacious in all settings

era vaccine was to increase the dose of the vaccine, and the one used for measles would be to delay administration of the vaccine until the titers of maternal antibodies waned. Preliminary studies conducted in South Africa indicate that the immune response to Rotarix improved when the vaccine schedule was changed from 6 and 10 weeks of age to 10 and 14 weeks of age—an effect attributed to the waning of maternal antibody [42]. Because the half-life of transplacental antibody in the infant is 3–4 weeks, this 4-week delay in vaccination correlates with a halving of titers in the infant at the time of the later dose.

With anticipation of the problem that maternal antibody might interfere with immunogenicity, a multi-center study has recently been concluded in Africa, in both a middle-income country (South Africa) [67] and a low-income country (Malawi). Two novel alterations in administration that were not previously tested for the licensed vaccine were used to potentially improve the efficacy of Rotarix in this trial. One arm of the study added a third dose administered at 14 weeks of age to the current schedule of 2 doses at 6 and 10 weeks of age, and the second arm changed administration of the 2-dose regimen from the 6- and 10-week routine immunization schedule of the WHO to a delayed schedule at 10 and 14 weeks of age; this schedule was specifically designed to overcome the potential impact of high titers of maternal antibody at the time of vaccination, based on a previous study [42]. Of note, both interventions included a dose at 14 weeks of age; therefore, if levels of maternal antibody are a contributing factor to decreased immunogenicity, administration of a later dose when maternal titers have waned should result in improved efficacy. Although a delay in vaccination could yield a major improvement in immune response and efficacy, it would require some substantial rethinking of the routine immunization schedule currently set by the WHO immunization program at 6 and 10 weeks of age. Many children do not receive their vaccination

on schedule; thus, these common but unplanned delays could substantially improve the effectiveness of the vaccine. However, such delays also increase the opportunity for natural infection early in life and its sequelae.

To determine whether breastfeeding at the time of vaccination might impair the infant's immune response, breastfeeding could be withheld for a period before and after the vaccine is administered, as is being done in field trials of enterotoxigenic *Escherichia coli* vaccines in Bangladesh (A. M. Svennerholm, personal communication). The exact time of transit of a rotavirus strain from the mouth to the intestine is not precisely known. Gastric emptying times estimated using the Carbon 13 octanoic breath test arrived at a half-time of 47 min [68]; however, these estimates might not be the same for infants in developing countries. Because infants aged 6–24 weeks feed every 120–180 min [69], a delay of 60 min before or after vaccination would allow assessment of the inhibitory effect that concomitant breastfeeding and vaccination might have on immunogenicity. It could also improve the depressed immune response to the vaccine found in Thai infants that was previously reported [45]. Other interventions could be targeted to assess improvements in host response that might be achieved by administering zinc, vitamin A, or a probiotic, such as *Lactobacillus* GG, at the time of vaccination. Each addition to the regimen makes vaccine delivery a greater challenge, but understanding where the problem may lie would allow a focus on the interventions that are critical.

LOOKING AHEAD

This review was stimulated by the preliminary observation from trials of Rotarix that the immune response to the oral rotavirus vaccines in key target populations in developing countries appeared to be suboptimal and might result in the vaccine having diminished efficacy. The question raised by these observations

would be, what level of vaccine efficacy would be required to declare the vaccine a success, and could this efficacy be improved by slight changes in the timing or manner of vaccination? The response to this question differs according to the group or person queried and the cost of vaccine used in a cost-effectiveness analysis. Because of the high mortality associated with rotavirus in many of these target countries, an efficacy <80% would be acceptable, but the response to an efficacy of 40%–60% would be unanswered. Furthermore, we have a test system for infants that could help improve the vaccine through a series of studies: delaying vaccination to avoid the inhibitory effect of maternal antibody, withholding breastfeeding at the time of vaccination, or administering zinc or a probiotic to the infant at the time of vaccination. Although the programmatic issues surrounding implementation of these strategies would need to be carefully considered, studying each of these interventions could help elucidate the mechanism of the weak immune response and provide a clear avenue for an effective intervention, in the event that ongoing efficacy trials show sub-optimal results.

These hypotheses rest on the assumption that the observed immune response will translate into a lower-than-expected efficacy; we will not know this for another 2–3 years for both African and Asian children. A recently released analysis of the trial of Rotarix in South Africa indicated that the vaccine had an efficacy of 77%; however, any subanalysis of differences between rural and urban areas was not reported [29, 69]. In contrast, Rotarix only prevented 49% of severe rotavirus disease in Malawi [29]. The population of Malawi is substantially poorer than that of South Africa, and the per capita income is significantly lower. Although seroconversion may be a poor predictor of vaccine efficacy, it reflects the processing of the vaccine virus in the gut, which increases the likelihood that the vaccine should prevent disease. Should the problem of weak immune responses be inherent with these live oral vaccines, other vaccine approaches should be pursued aggressively as insurance and backup for the current leading candidates. Live oral vaccines derived from neonatal strains, such as the Australian strain RV3 and the India strain 116E, each have the advantage that they grow well in the presence of maternal antibody because of a single substitution of the VP4 gene of a human rotavirus with a less common or bovine VP4 variant. These vaccines are in clinical development. Alternatively, parenteral vaccines derived from inactivated virus or virus-like particles or subunit vaccines might avoid the intestinal processing of these living strains but provide the needed protection [70–73]. Investment in these approaches may provide added insurance against major delays should efficacy be found to be suboptimal. While the world awaits the results of trials now in progress, we need to think ahead and plan for ways to address

these problems now so that we do not suffer added delays in the future.

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